

**BIOLOGICAL INTEGRITY OF  
LITTLE DRY CREEK  
BASED ON THE STRUCTURE AND COMPOSITION OF  
THE BENTHIC ALGAE COMMUNITY**

**Prepared for:**

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## Summary

On July 29, 2003, periphyton samples were collected from three sites on Little Dry Creek near Jordan, Montana for the purpose of assessing whether this stream is water-quality limited and in need of TMDLs. The samples were collected following MDEQ standard operating procedures, processed and analyzed using standard methods for periphyton, and evaluated following modified USEPA rapid bioassessment protocols for wadeable streams.

**All three sites exhibited minor impairment but full support of aquatic life uses.** The dominant alga at all three sites was *Rhizoclonium*, a free-living and mat forming filamentous green alga that often causes problems in standing waters in the western United States. Diatoms and other genera of filamentous green algae were also common at all three sites.

Diatom metrics indicate minor impairment from organic loading and sedimentation at the **Cohagen and Uall Creek** sites, but otherwise excellent biological integrity for a prairie stream. The Cohagen site was the only site that supported free-living nitrogen-fixing cyanobacteria (*Tolypothrix*) and this site also had the largest percentage of nitrogen-fixing diatoms. The abundance of nitrogen-fixing algae indicates that biologically available inorganic nitrogen was probably in short supply (limiting) relative to phosphorus. The diatom assemblage at Cohagen consisted mostly of facultative nitrogen heterotrophs that can use organic matter as a source of nitrogen. The Cohagen and Uall Creek sites shared about 60% of their diatom assemblages, indicating that they were quite similar both floristically and ecologically.

Diatom metrics and ecological attributes at the **Van Norman** site indicate significantly improved water quality compared to the two upstream sites. The pollution index here was just below the threshold for minor impairment and the sedimentation index was well below the threshold for minor impairment. The predominance of pollution sensitive species and other indicators show that this site had significantly lower concentrations of organic matter and higher concentrations of dissolved oxygen than the Cohagen and Uall Creek sites. The Van Norman site supported the smallest percentage of nitrogen-fixing diatoms, indicating that biologically available inorganic nitrogen was probably not in short supply relative to phosphorus. This site shared only about 30% of its diatom assemblage with the two upstream sites, indicating that it was quite dissimilar from these two sites both floristically and ecologically.

## Introduction

This report evaluates the biological integrity<sup>1</sup>, support of aquatic life uses, and probable causes of stress or impairment to aquatic communities in Little Dry Creek near Jordan in east central Montana. The purpose of this report is to provide information that will help the State of Montana determine whether Little Dry Creek is water-quality limited and in need of TMDLs.

The federal Clean Water Act directs states to develop water pollution control plans (Total Maximum Daily Loads or TMDLs) that set limits on pollution loading to water-quality limited waters. Water-quality limited waters are lakes and stream segments that do not meet water-quality standards, that is, that do not fully support their beneficial uses. The Clean Water Act and USEPA regulations require each state to (1) identify waters that are water-quality limited, (2) prioritize and target waters for TMDLs, and (3) develop TMDL plans to attain and maintain water-quality standards for all water-quality limited waters.

Evaluation of aquatic life use support in this report is based on the species composition and structure of periphyton (benthic algae, phytobenthos) communities at three sites that were sampled on July 29, 2003. Periphyton is a diverse assortment of simple photosynthetic organisms called algae that live attached to or in close proximity of the stream bottom. Some algae form long filaments or large colonies that are conspicuous to the unaided eye. But most, including the ubiquitous diatoms, can be seen and identified only with the aid of a microscope. The periphyton community is a basic biological component of all aquatic ecosystems. Periphyton accounts for much of the primary production and biological diversity in Montana streams (Bahls et al. 1992). Plafkin et al. (1989) and Barbour et al. (1999) list several advantages of using periphyton in biological assessments.

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<sup>1</sup> *Biological integrity* is defined as “the ability of an aquatic ecosystem to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitats within a region” (Karr and Dudley 1981).

## **Project Area and Sampling Sites**

The project area is located in Garfield County in east central Montana. Little Dry Creek heads west of Cohagen at an elevation of about 3,500 feet, then flows east and north to meet Big Dry Creek about 25 miles east of Jordan and just north of Montana Highway 200 near the Van Norman community. The combined flows of Little Dry and Big Dry Creeks then continue north into the Big Dry Arm of Fort Peck Reservoir on the Missouri River.

The project area is within the Northwestern Great Plains Ecoregion (Woods et al. 1999). The surface geology of the area consists of sandstone and shales of the Hell Creek Formation and rocks of the coal-bearing Fort Union Formation (Renfro and Feray 1972). The climate is semiarid and continental, with cold winters and hot, dry summers. Upland vegetation is predominantly mixed grassland (USDA 1976). The main land use is livestock grazing.

Periphyton samples were collected at three sites on Little Dry Creek (Table 1). Elevations at the sampling sites range from about 3,000 feet at Cohagen to about 2,400 feet at the Van Norman School.

## **Methods**

Periphyton samples were collected following standard operating procedures of the MDEQ Planning, Prevention, and Assistance Division. Using appropriate tools, microalgae were scraped, brushed, or sucked from natural substrates in proportion to the importance of those substrates at each study site. Macroalgae were picked by hand in proportion to their abundance at the site. All collections of microalgae and macroalgae were pooled into a common container and preserved with Lugol's (IKI) solution.



The samples were examined to estimate the relative abundance and rank by biovolume of diatoms and genera of soft (non-diatom) algae according to the method described in Bahls (1993). Soft algae were identified using Smith (1950), Prescott (1962, 1978), John et al. (2002), and Wehr and Sheath (2003). These books also served as references on the ecology of the soft algae, along with Palmer (1969, 1977).

After the identification of soft algae, the raw periphyton samples were cleaned of organic matter using sulfuric acid, potassium dichromate, and 3% hydrogen peroxide. Then, permanent diatom slides were prepared using Naphrax™, a high refractive index mounting medium, following *Standard Methods for the Examination of Water and Wastewater* (APHA 1998). Approximately 350 diatom cells (700 valves) were counted at random and identified to species. The following were the main taxonomic references for the diatoms: Krammer and Lange-Bertalot 1986, 1988, 1991a, 1991b; Lange-Bertalot 2001; Krammer 2002. Diatom naming conventions followed those adopted by the Academy of Natural Sciences for USGS NAWQA samples (Morales and Potapova 2000). Van Dam et al. (1994) was the main ecological reference for the diatoms.

The diatom proportional counts were used to generate an array of diatom association metrics. A metric is a characteristic of the biota that changes in some predictable way with increased human influence (Barbour et al. 1999). Diatoms are particularly useful in generating metrics because there is a wealth of information available in the literature regarding the pollution tolerances and water quality preferences of common diatom species (e.g., Lowe 1974, Beaver 1981, Lange-Bertalot 1979, 1996, Van Dam et al. 1994).

Values for selected diatom metrics were compared to biocriteria (numeric thresholds) developed for streams in the Great Plains ecoregions of Montana (Table 2). These criteria are based on the distribution of metric values measured in least-impaired reference streams (Bahls et al. 1992) and metric values measured in streams that are known to be impaired by various sources and causes of pollution (Bahls 1993). The biocriteria in Table 2 are valid only for samples collected during the summer field season (June 21-September 21).

The criteria in Table 2 distinguish among four levels of stress or impairment and three levels of aquatic life use support: (1) no impairment or only minor impairment (full support); (2) moderate impairment (partial support); and (3) severe impairment (nonsupport). These impairment levels correspond to excellent, good, fair, and poor biological integrity, respectively.

## **Quality Assurance**

Several steps were taken to assure that the study results are accurate and reproducible. Upon receipt of the samples, station and sample attribute data were recorded in the Montana Diatom Database and the samples were assigned a unique number, e.g., 3007-01. The first part of this number (3007) designates the sampling site (Little Dry Creek at Cohagen) and the second part (01) designates the number of periphyton samples that have been collected at this site for which data have been entered into the Montana Diatom Database.

Sample observations and analyses of soft (non-diatom) algae were recorded in a lab notebook along with information on the sample label. A portion of the raw sample was used to make duplicate diatom slides. The slide used for the diatom proportional count will be deposited in the Montana Diatom Collection at the University of Montana Herbarium (MONTU) in Missoula. The duplicate slide will be retained in Helena at the offices of *Hannaea*. Diatom proportional counts have been entered into the Montana Diatom Database.

## **Results and Discussion**

Results are presented in Tables 3, 4, and 5, which are located near the end of this report following the references section. Appendix A consists of a series of diatom reports, one for each sample. Each diatom report contains an alphabetical list of diatom species and their percent abundances, and values for 65 different diatom metrics and ecological attributes.

## Sample Notes

**Little Dry Creek at Cohagen.** The sample from this site was anoxic and black with  $\text{H}_2\text{S}$ , but it was cleared with  $\text{H}_2\text{O}_2$  and was usable. Fine particulate organic matter and fine to coarse inorganic sediment were very abundant in the sample.

**Little Dry Creek near mouth of Uall Creek.** The sample from this site was also anoxic and black with  $\text{H}_2\text{S}$ , but it was also cleared with  $\text{H}_2\text{O}_2$  and usable. Fine particulate organic matter and fine inorganic sediment were abundant in the sample.

**Little Dry Creek near Van Norman School.** The sample from this site was anoxic, putrid, and opaque black with  $\text{H}_2\text{S}$ . Algal features were obscured but clear enough for positive identification. Fine particulate organic matter was very abundant.

## Non-Diatom Algae (Table 3)

All three sites had similar non-diatom assemblages dominated by the filamentous green alga *Rhizoclonium*. *Rhizoclonium* and *Cladophora* are mat-forming green algae that often cause problems in standing waters in the western United States (Wehr and Sheath 2003). Algae interfere with water uses—e.g., fishing, swimming, boating, irrigation—only when standing crops are excessive. Mat-forming filamentous algae are normal components of many aquatic ecosystems, including prairie streams, and there is no evidence from this study that standing crops of these algae in Little Dry Creek are excessive. Also, criteria have not been established for determining when algal growth in prairie streams is excessive.

The filamentous and nitrogen-fixing cyanobacterium *Tolypothrix* was abundant and ranked second in biovolume in Little Dry Creek at Cohagen. *Tolypothrix* requires cooler waters than most other cyanobacteria. This site also supported two other genera of filamentous green algae besides *Rhizoclonium* (Table 3). The co-occurrence of *Tolypothrix* with filamentous green algae is not unusual. Diatoms were abundant and ranked third in biovolume at all three sites.



*Tolypothrix* was absent from the middle and lower sites on Little Dry Creek, perhaps indicating that these sites had warmer waters and/or more biologically available nitrogen than the upstream site. Little Dry Creek near the mouth of Uall Creek and Little Dry Creek near the Van Norman School supported the same three genera of filamentous green algae as the upstream site. In addition, *Cladophora*, another mat-forming and potential problem alga, was common at the Van Norman site (Table 3).

## Diatoms (Table 4)

Of the 15 major diatom species in Little Dry Creek, 2 are sensitive to organic pollution (class 3) and 2 are most tolerant of organic pollution (class 1). Sensitive and most tolerant species were abundant at all three sites (Table 4). The remaining 11 major species are somewhat tolerant of organic pollution and these (class 2) species were also abundant at all three sites. All three sites had excellent diatom species richness and diversity for a prairie stream.

**Little Dry Creek at Cohagen.** Diatom metrics indicate **minor impairment** from organic loading and sedimentation at this site but otherwise excellent biological integrity and **full support of aquatic life uses** (Table 4). The dominant diatom species at this site were *Nitzschia amphibia* and *Nitzschia inconspicua*. Both of these species are facultative nitrogen heterotrophs that prefer eutrophic, alkaline, and brackish-fresh waters and need periodically elevated concentrations of organically bound nitrogen (Van Dam et al. 1994).

Of the three sites, the Cohagen site also supported the largest percentage of diatoms in the order Rhopalodiales (Table 4). Diatoms in the order Rhopalodiales harbor endophytic nitrogen-fixing symbionts (cyanobacteria). The large percentage of Rhopalodiales at Cohagen, coupled with an abundance of the nitrogen-fixing cyanobacterium *Tolypothrix*, indicates that biologically available inorganic nitrogen was probably in short supply (limiting) relative to phosphorus. With an abundance of both autotrophic and heterotrophic algae at this site, any increase in organic or inorganic nitrogen would likely result in an increase in algal growth.

**Little Dry Creek near mouth of Uall Creek.** This site supported somewhat larger percentages of tolerant species and motile species, resulting in a smaller pollution index and a larger siltation index than the Cohagen site (Table 4). Nevertheless, diatom metrics indicated only **minor impairment and full support of aquatic life uses**. This site shared about 60% of its diatom assemblage with the Cohagen site, which indicates that the two sites were virtually identical, both floristically and ecologically. Both the Cohagen site and the Uall Creek site had exceptional diatom species richness, diversity, and evenness for a prairie stream.

**Little Dry Creek near Van Norman School.** This site had the highest pollution index, the lowest sedimentation index, and the best biological integrity of all three sites. The large pollution index, due mostly to dominance by the pollution-sensitive species *Achnanthes minutissimum*, was just below the threshold (2.25) for no impairment and excellent biological integrity. The percentage of motile diatoms at Van Norman in the genera *Navicula*, *Nitzschia*, and *Surirella* was well below the threshold (50.0) for minor impairment from sedimentation in prairie streams. A strict comparison of diatom metric values in Table 4 to biocriteria in Table 2 would indicate that the Van Norman site was subject to **minor impairment from organic loading and biological or physical disturbance**, which may have been natural in origin.

The Van Norman site shared only 34% of its diatom assemblage with the Uall Creek site (Table 4) and only 28% with the Cohagen site. Hence, of the three sites this one was the most unique in terms of both floristics and ecology. Flow regime sets this site apart from the upper sites. Little Dry Creek flows all the time at Van Norman, whereas the upper sites consist of a series of pools (Rebecca Ridenour, MDEQ, personal communication).

Of the three sites, Van Norman supported the lowest percentage of diatoms in the order Rhopalodiales, indicating that inorganic nitrogen was probably more available here than it was upstream. This site also had excellent diatom species richness and diversity for a prairie stream.

## **Modal Categories (Table 5)**

Several ecological attributes were selected from the diatom reports in the appendix and modal categories of these attributes were extracted to characterize water quality tendencies at the three sites on Little Dry Creek (Table 5).

Modal categories were very similar for the two upstream sites. Most diatoms at these sites were highly motile, alkaliphilous, eutraphentic species that indicate waters with 25-70% saturation of dissolved oxygen and 4-13 mg/L BOD<sub>5</sub> (Van Dam et al. 1994). BOD is a measure of organic loading in a body of water. Additional organic loading may result in more growth of aquatic bacteria, fungi, and heterotrophic algae and in lower concentrations of dissolved oxygen. The intermediate level of BOD loading (saprobity) at the two upstream sites is less than what would be discharged by a sewage treatment plant, but more than what would be present in a stream where biodegradable organic matter has been mostly decomposed.

Most diatoms at Cohagen were facultative nitrogen heterotrophs that require only moderate (above 50% saturation) dissolved oxygen. The modal category for nitrogen uptake and oxygen demand at the Uall Creek site was "not classified".

Modal categories at Van Norman were significantly different from those at the two upstream sites (Table 5). Most diatoms at Van Norman were only moderately (not highly) motile and indicated circumneutral (not alkaline) waters. Most diatoms here were nitrogen autotrophs that require inorganic nitrogen (nitrates and ammonia) rather than preformed organic matter as a source of nitrogen. The majority of diatoms at Van Norman requires continuously high concentrations of dissolved oxygen and indicates smaller amounts of BOD<sub>5</sub> (2-4 mg/L). Most diatoms at Van Norman tolerate a wide range of trophic conditions ranging from oligotrophic to eutrophic (Table 5).

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Table 1. Location of MDEQ periphyton sampling stations on Little Dry Creek near Jordan, Montana in 2003.

Station	Montana DEQ Station Code	<i>Hannaea</i> Sample Number	Latitude	Longitude	Sample Date
Little Dry Creek at Cohagen	M29LDRYC03	3007-01	47 03 17	106 36 46	7/29/03
Little Dry Creek near mouth of Uall Creek	M29LDRYC02	3006-01	47 04 31	106 16 59	7/29/03
Little Dry Creek near VanNorman School	M29LDRYC01	3005-01	47 20 30	106 21 47	7/29/03

Table 2. Diatom association metrics used by the State of Montana to evaluate biological integrity in **prairie** streams: references, range of values, expected response to increasing impairment or natural stress, and criteria for rating levels of biological integrity. The lowest rating for any one metric is the rating for that site.

Biological Integrity/ Impairment or Stress/ Use Support	No. of Species Counted <sup>1</sup>	Diversity Index <sup>2</sup> (Shannon)	Pollution Index <sup>3</sup>	Siltation Index <sup>4</sup>	Disturbance Index <sup>5</sup>	% Dominant Species <sup>6</sup>	Similarity Index <sup>7</sup>
Excellent/None Full Support	>39	>3.99	>2.25	<50.0	<25.0	<25.0	>59.9
Good/Minor Full Support	30-39	3.00-3.99	1.76-2.25	50.0-69.9	25.0-49.9	25.0-49.9	40.0-59.9
Fair/Moderate Partial Support	20-29	2.00-2.99	1.25-1.75	70.0-89.9	50.0-74.9	50.0-74.9	20.0-39.9
Poor/Severe Nonsupport	<20	<2.00	<1.25	>89.9	>74.9	>74.9	<20.0
References	Bahls 1979 Bahls 1993	Bahls 1979	Bahls 1993	Bahls 1993	Barbour et al. 1999	Barbour et al. 1999	Whittaker 1952
Range of Values	0-100+	0.00-5.00+	1.00-3.00	0.0-90.0+	0.0-100.0	~5.0-100.0	0.0-100.0
Expected Response	Decrease	Decrease	Decrease	Increase	Increase	Increase	Decrease

<sup>1</sup>Based on a proportional count of 400 cells (800 valves)

<sup>2</sup>Base 2 [bits] (Weber 1973)

<sup>3</sup>Composite numeric expression of the pollution tolerances assigned by Lange-Bertalot (1979) to the common diatom species

<sup>4</sup>Sum of the percent abundances of all species in the genera *Navicula*, *Nitzschia*, and *Surirella*

<sup>5</sup>Percent abundance of *Achnanthes minutissimum* (synonym: *Achnanthes minutissima*)

<sup>6</sup>Percent abundance of the species with the largest number of cells in the proportional count

<sup>7</sup>Percent Community Similarity (Whittaker 1952)

Table 3. Relative abundance of cells and ordinal rank by biovolume of diatoms (Division Bacillariophyta) and genera of non-diatom algae in periphyton samples collected from Little Dry Creek in 2003: d = dominant; a = abundant; f = frequent; c = common; o = occasional; r = rare.

Taxa	At Cohagen M29LDRYC03	Near Mouth of Uall Creek M29LDRYC02	Near Van Norman School M29LDRYC01
<b>Cyanophyta</b>			
<i>Tolypothrix</i>	abundant/2		
<b>Chlorophyta</b>			
<i>Cladophora</i>			common/4
<i>Oedogonium</i>	abundant/4	frequent/4	occasional/5
<i>Rhizoclonium</i>	dominant/1	dominant/1	dominant/1
<i>Spirogyra</i>	common/5	frequent/2	abundant/2
<b>Bacillariophyta</b>			
	frequent/3	abundant/3	abundant/3
<b>No. Non-Diatom Genera</b>	4	3	4

Table 4. Percent abundance of major diatom species<sup>1</sup> and values of selected diatom association metrics for periphyton samples collected from Little Dry Creek in 2003. Underlined values indicate minor stress; **bold values** indicate moderate stress; underlined and bold values indicate severe stress; all other values indicate no stress and full support of aquatic life uses when compared to criteria for mountain streams in Table 2 and plains streams in Table 3. Stress may be natural or anthropogenic (see text)

Species/Metric	PTC <sup>2</sup>	At Cohagen M29LDRYC03	Near Mouth of Uall Creek M29LDRYC02	Near Van Norman School M29LDRYC01
<i>Achnanthisidium minutissimum</i>	3	2.18	7.65	27.15
<i>Amphipleura pellucida</i>	2		0.31	3.28
<i>Diploneis puella</i>	2	6.86	5.35	5.93
<i>Encyonopsis microcephala</i>	2			8.42
<i>Encyonopsis subminuta</i>	2			3.12
<i>Navicula durrenbergiana</i>	1			9.98
<i>Navicula subrhynchocephala</i>	2	3.12	3.52	0.94
<i>Nitzschia amphibia</i>	2	13.42	4.59	
<i>Nitzschia elegantula</i>	2	0.62	6.88	0.94
<i>Nitzschia inconspicua</i>	2	11.23	8.56	1.09
<i>Nitzschia palea</i>	1	2.50	6.73	3.59
<i>Nitzschia paleacea</i>	2	5.77	6.12	0.31
<i>Nitzschia pura</i>	2	0.31	4.13	
<i>Rhoicosphenia abbreviata</i>	3	5.30	3.67	2.81
<i>Rhopalodia gibba</i>	2	4.68	0.76	0.16
Number of Species Counted		63	57	55
Shannon Species Diversity		5.01	5.00	4.42
Pollution Index		<u>2.01</u>	<u>1.93</u>	<u>2.24</u>
Siltation Index		<u>61.00</u>	<u>66.36</u>	32.29
Disturbance Index		2.18	7.65	<u>27.15</u>
Percent Dominant Species		13.42	8.56	<u>27.15</u>
Percent Rhopalodiales		8.27	1.38	0.16
Similarity Index <sup>3</sup>			59.55	34.41

<sup>1</sup>A major diatom species accounts for 3.0% or more of the cells at one or more stations in a sample set.

<sup>2</sup>(Organic) Pollution Tolerance Class (Lange-Bertalot 1979): 1 = most tolerant; 2 = tolerant; 3 = sensitive.

<sup>3</sup>Percent Community Similarity (Whittaker 1952) when compared to the diatom assemblage at the adjacent upstream station

Table 5. Modal categories for selected ecological attributes of diatom species in Little Dry Creek. Categories that represent significant departures from other sites are given in **bold face type**.

Ecological Attribute	At Cohagen M29LDRYC03	Near Mouth of Uall Creek M29LDRYC02	Near Van Norman School M29LDRYC01
Motility <sup>1</sup>	Highly Motile	Highly Motile	<b>Moderately Motile</b>
pH <sup>2</sup>	Alkaliphilous	Alkaliphilous	<b>Circumneutral</b>
Salinity <sup>2</sup>	Fresh	Fresh	Fresh
Nitrogen Uptake <sup>2</sup>	Facultative Nitrogen Heterotrophs	Not Classified	<b>Nitrogen Autotrophs (high organics)</b>
Oxygen Demand <sup>2</sup>	Moderate	Not Classified	<b>Continuously High</b>
Saprobity <sup>2</sup>	alpha-Mesosaprobous	alpha-Mesosaprobous	<b>beta-Mesosaprobous</b>
Trophic State <sup>2</sup>	Eutraphentic	Eutraphentic	<b>Variable</b>

<sup>1</sup>Dr. R. Jan Stevenson, Michigan State University, digital communication.

<sup>2</sup>Van Dam et al. 1994





